ehp

LESSON:

Triple Washed, Twice Shy?

Summary Students read a brief article about food safety and prepackaged produce. Then they conduct an experiment to determine whether washing prepackaged produce reduces bacteria levels.

Lesson Type **Experiment**—Students collect, manipulate, and/or summarize data from an experiment they conduct.

***EHP* Article** "Triple Washed, Twice Shy?"
Environ Health Perspect 116:A477 (2008)
http://www.ehponline.org/docs/2008/116-11/forum.html#beat

Objectives By the end of this lesson, students should be able to
- design an experiment to test whether washing "prewashed" salads reduces the number of microorganisms present on the produce
- perform bacterial culturing technique
- analyze experimental results
- describe types of bacteria observed

Class Time 2–3 class periods for activity, up to 5–6 days for observation

Grade Level Middle school, high school, college

Subjects Addressed Biology, General Science

Aligning with Standards

SKILLS USED OR DEVELOPED

- Classification
- Communication (note-taking, oral, written—including summarization)
- Comprehension (listening, reading)
- Computation
- Critical thinking and response
- Experimentation (design, conduct, data analysis)
- Graphing
- Manipulation
- Observation
- Research
- Tables and figures (creating, reading)

SPECIFIC CONTENT ADDRESSED

- Foodborne illness
- Microbiology

NATIONAL SCIENCE EDUCATION STANDARDS MET

Science Content Standards

Unifying Concepts and Processes Standard
- Systems, order, and organization
- Change, constancy, and measurement
- Evidence, models, and explanation
- Form and function

Science as Inquiry Standard
- Abilities necessary to do scientific inquiry
- Understanding about scientific inquiry

Life Science Standard
- The cell
- Matter, energy, and organization in living systems
- Interdependence of organisms
- Behavior of organisms

Science in Personal and Social Perspectives Standard
- Personal and community health
- Science and technology in local, national, and global challenges

History and Nature of Science Standard
- Science as a human endeavor
- Nature of scientific knowledge

Prepping the Lesson (20–30 minutes)

INSTRUCTIONS

1. Download the *EHP* article "Triple Washed, Twice Shy?" at http://www.ehponline.org/docs/2008/116-11/forum.html#beat.
2. Review the Background Information, Instructions, Assessing the Lesson, and Student Instructions for this lesson.
3. Make copies of the article and the Student Instructions.
4. Collect the other materials listed below.
5. Open the bag of salad and use sterile tongs to place 6–8 leaves in a new plastic zipper-locked sandwich bag. Each group of 3–5 students will need 1 bag of leaves. Keep the salad wrapper to show students.
6. Place 6–8 sterile cotton swabs in a new plastic zipper-locked sandwich bag, or obtain individually sealed cotton swabs (preferred). Each group of 3–5 students will need 1 bag of 6–8 swabs *or* 6–8 individually wrapped swabs.
7. This activity can be done with one of two culture media: agar or potato slices. Decide which medium you will use, and prepare the culture medium.
   a. If you are using agar:
      1) Prepare the solution as instructed on the agar package, and pour into Petri dishes.
      2) Each group of 3–5 students will need 3 Petri dishes.
   b. If you are using potatoes:
      1) Do not peel the potatoes. Wash and slice each potato into slices approximately 2 cm thick.
      2) Put as many slices as will fit in a beaker or pot, and fill with water.
      3) Heat the water and potatoes to boiling for 4 minutes. Then pour out most of the water, leaving just enough to keep the slices from drying out.
      4) Sterilize the tongs by heating them in a Bunsen burner until the tines turn red. Tongs cleaned in a dishwasher are also relatively sterile, but it is best to sterilize the tongs using heat.
      5) Transfer 1 potato slice to a sterile Petri dish and cover the dish. Let the slice cool to room temperature. If you are preparing the potatoes the night before, you can place cooked potatoes in a sterile plastic container such as a plastic bag or Tupperware bowl, then refrigerate overnight. Potatoes can be transferred to Petri dishes the following day. Each group of 3–5 students will need 3–6 Petri dishes.

MATERIALS

per student

- 1 copy of the article "Triple Washed, Twice Shy?" preferably in color
- 1 copy of the Student Instructions
- 1 copy of the Culturing Instructions
- 1 copy of the 3-page Record Sheet

per group of 3–5 students

- 1 bag of 6–8 sterile cotton swabs
- 1 bag of 6–8 salad leaves
- 1 grease pencil or marker
- 1 stopwatch
- 1 clean plastic cup or beaker to wet cotton swabs
- 3–6 additional clean plastic cups or beakers if groups wish to soak salad leaves
- sterile tweezers
- safety goggles and gloves
- masking tape
- paper towels
- materials to clean the produce (lemon juice, white vinegar, apple cider vinegar, produce wash, etc.)
- 3–6 sterile Petri dishes
- culture medium
  - If you are using agar:
    - agar
    - Bunsen burner
  - If you are using potato slices:
    - potatoes (1 potato provides approximately 4 to 6 2-cm slices)
    - knife

- cutting board
- pot or beaker
- hotplate or stovetop range
- kitchen mitt or hot glove
- clean plastic zipper-lock sandwich bags
- sterile tongs

for optional wet-mount slide activity

- 1 copy of the Preparing a Wet-Mount Slide Instructions
- 1 microscope
- 1 clean microscope slide
- 1 coverslip
- 1 pipette
- 2 sterile probes or toothpicks

per class

- sink with running water
- hand soap
- 1–2 bags prepackaged salad
- tap or distilled water
- disinfectant to clean workstations

VOCABULARY

- agar
- aseptic technique
- bacteria
- colony
- culture
- culture medium
- differential media
- filamentous
- foodborne illness
- hemolytic
- microorganism
- morphology
- pathogen
- Petri dish
- pure culture
- selective media
- sterile

BACKGROUND INFORMATION

Microorganisms, including bacteria and viruses, are everywhere. Bacteria are vital to life as we know it. Some bacteria produce chemical energy through photosynthesis, whereas others decompose organic materials to recycle nutrients through the ecosystem. Bacteria are also used for many human purposes, such as processing foods and making new drugs. However, some microorganisms are pathogens that can cause disease in humans and other animals.

In the past few years, a number of outbreaks of foodborne illness have been associated with eating unwashed prepackaged fruits and vegetables. In 2006, for example, an outbreak of *Escherichia coli* was linked to prepackaged spinach. Current recommendations suggest that any prepackaged produce labeled "washed," "triple-washed," or "ready to eat" need not be washed before use, because if pathogens remain on prepared salads after commercial washing, further washing is not likely to remove them (Palumbo et al. 2007). Contaminants also may be introduced during the preparation of prepackaged fruits and vegetables, especially if produce is prepared with unclean hands or surfaces.

In this lesson, students will explore different methods of washing produce and then culture samples. Selective media contain ingredients that support the growth of one type of microorganism over another. For example, some media (such as MacConkey agar) inhibit the growth of gram-positive bacteria. If you do not have access to any selective media, this experiment can be done with agar or potatoes.

References

Centers for Disease Control and Prevention. 2005. Foodborne illness frequently asked questions. http://www.cdc.gov/ncidod/dbmd/diseaseinfo/files/foodborne_illness_FAQ.pdf

Miller KR, Levine JS. 2006. Biology. Upper Saddle River, NJ: Prentice Hall.

Palumbo MS, Gorny JR, Gombas DE, Beuchat LR, Bruhn CM, Cassens B, et al. 2007. Recommendations for handling fresh-cut leafy green salads by consumers and retail foodservice operators. Food Protection Trends 27:892–898.

Prescott LM, Harley JP, Klein DA. 2002. Microbiology, 5th ed. New York, NY: McGraw-Hill.

Rice University. MedMyst: medical mysteries on the web. http://medmyst.rice.edu/index.html

RESOURCES

Environmental Health Perspectives, Environews by Topic page. http://ehp.niehs.nih.gov/. Choose Food Safety and Regulation

American Society of Microbiology. Microbe World. http://www.microbeworld.org/findex.aspx

Dennis Kunkel Microscopy. http://www.denniskunkel.com/index.php

Partnership for Food Safety Education. FIGHT BAC! Keep Food Safe From Bacteria. http://www.fightbac.org/index.php

Science Buddies. Interpreting Plates (provides general information about classifying the form, elevation, and margin of bacterial cultures). http://www.sciencebuddies.org/science-fair-projects/project_ideas/MicroBio_Interpreting_Plates.shtml

Todar's Textbook of Bacteriology, http://www.textbookofbacteriology.net/; *Clostridia* spp., http://textbookofbacteriology.net/clostridia.html; *E. coli* spp., http://textbookofbacteriology.net/e.coli.html; *Salmonella* spp., http://www.textbookofbacteriology.net/salmonella.html; *Listeria* spp., http://textbookofbacteriology.net/Listeria.html

U.S. Department of Agriculture. Food Safety Education. http://www.fsis.usda.gov/food_safety_education/index.asp

U.S. Food and Drug Administration and National Science Teachers Assocation. Science and Our Food Supply (free curriculum supplement dealing with food safety). http://www.foodsafety.gov/~fsg/teach.html

U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition. Gateway to Government Food Safety Information. http://www.foodsafety.gov/foodsafe.html

Implementing the Lesson

INSTRUCTIONS

1. Have the students read the article "Triple Washed, Twice Shy?" and the information in Step 2 of the Student Instructions. Address any questions or clarify vocabulary as needed.
2. Pass the wrapper from the prepackaged salad around the class, and review the label with the students. Instruct them to record the label information in Step 3 of the Student Instructions.
3. Ask the students if they typically wash fresh fruits or vegetables before eating them. Call on one or two students to describe how they clean fruits and vegetables. You may wish to explain how heat, acidity, and other elements of cleaning kill microbes.
4. Divide the class into groups of 3–5 students each. Review Step 4 of the Student Instructions with the class, and ask them to write down how they would wash fresh fruits and vegetables. Prompt students to list some important factors to consider, such as:
   - washing time (e.g., have students hypothesize how a longer wash time might impact the presence of bacteria)
   - washing substance, such as water, soap, vinegar, lemon juice, produce wash (e.g., have students consider how soap works compared with vinegar or lemon juice, and how each material might affect the presence of bacteria—soap removes dirt to which bacteria may adhere whereas vinegar and lemon juice kill the bacteria)
   - water temperature (hot versus cold)
   - washing process (rubbing versus soaking)
5. Tell the groups they will be designing an experiment to see if washing prepackaged salads has an effect on the number of microorganisms present (Step 5 of the Student Instructions). Advise students to select 1 variable from the steps they wrote down in Step 4. Each variable should have at least 2 experimental conditions. For example, if students want to look at washing time, they should have 2 different time periods, such as 10 seconds and 20 seconds. If students want to look at washing substance, they should look at 2 different conditions with a single substance, such as 2 different amounts of vinegar mixed in water. Inform students that cleaning produce in a certain way can affect the integrity or "quality" of the food (e.g., hot water may begin cooking the spinach and make it too mushy to use in a salad).
6. Distribute the Culturing Instructions and the Record Sheet to each group, and review them as a class. Instruct students to write their variables on the appropriate page of the Record Sheet (10 seconds under Test 1, etc.). Demonstrate the sampling procedure with students, and emphasize use of aseptic techniques. Tell students they need to exercise caution when working with microorganisms. Students should follow all laboratory safety rules, including keeping long hair tied back and not consuming food or beverages in the lab. Students should wash their hands before and after conducting the experiment. Gloves should be worn to protect students from any harmful bacteria that may be present as well as to reduce the risk of contamination of the experiment by bacteria from the students' hands. Goggles should be worn to protect students' eyes from substances that may cause irritation or injury (e.g., vinegar or lemon juice). Petri dishes should remain closed at all times except when swabbing the medium.

7. Students can complete Steps 6 and 7 of the Student Instructions as a group. Remind students that every experiment needs to have a control. For this experiment, a control would be a sample taken from an unwashed piece of salad. Circulate among groups to review each group's protocol and offer feedback.

   NOTE: If you are using potatoes as your culture medium, you will need an additional control to see if there are any bacteria already present on the potato. Use sterile tongs to place a "blank" (unswabbed) potato slice in a sterile Petri dish. Create this control while students are conducting their experiment, and ask them why it is important to have an additional control. Keep this secondary control with the other Petri dishes, and have students observe it along with their results.

8. Distribute Petri dishes and cotton swabs to each group. Show students any available cleaning materials, such as lemon juice or vinegar. Have students conduct the experiment (Step 8 of the Student Instructions). Students should take multiple samples. If using agar, have students divide the plate in half either by visualizing a line across the Petri dish or drawing a line using a grease pencil. For example, to look at washing times, students would sample 2 salad leaves washed for 10 seconds (1 Petri dish), 2 salad leaves washed for 20 seconds (1 Petri dish), and 2 control leaves (1 Petri dish). If using potato slices, students may need to 1 dish for each sample, depending on the size of the slices.

9. Once students collect their samples, place them away from direct sunlight to minimize mold growth and protect bacteria from UV light and excessive heat. Supervise while students clean their work areas. Autoclave any lab equipment if necessary.

10. Have groups observe the plates each day on Days 3–6 (Step 9 of the Student Instructions). Students should count the number of colonies they observe, as well as draw and describe the colonies in their observation notes. They should be sure to differentiate between different-looking colonies. NOTE: If there are lots of bacteria present, a smear will be seen rather than individual colonies. A helpful guide to interpreting colonies is available at http://www.sciencebuddies.org/science-fair-projects/project_ideas/MicroBio_Interpreting_Plates.shtml.

11. After Day 6, have students complete Steps 10–12 of the Student Instructions within their groups. Have students present their experimental design and results to the class.

    NOTE: If you have the time and equipment, groups may prepare a wet-mount slide of a colony and examine it under a microscope. You can also prepare a slide and set it up under a microscope for students to observe. See the Preparing a Wet-Mount Slide Instructions handout for more information.

12. Students can complete Step 14 of the Student Instructions individually for homework or as an in-class assignment.

13. Collect used Petri dishes. Use an autoclave to sterilize equipment, if available. If you wish to reuse the Petri dishes, then do the following:
    - Put on safety gloves.
    - Open the Petri dishes, and empty medium into a plastic garbage bag. Dispose of the bag in the trash or biohazardous waste container, if available.
    - Run the Petri dishes through an autoclave or dishwasher.
    - You can also wash the Petri dishes by filling a dishpan with soapy water and washing the dishes using a sponge or brush. Rinse dishes, and set to dry. Apply rubbing alcohol to the inside surfaces of the Petri dishes before next use.

Notes & Helpful Hints

- If Petri dishes are kept at room temperature, bacterial growth should be seen around Day 2 or 3. If you begin this experiment on Friday, then you will see growth by Monday.
- You can encourage different groups of students to test different variables. These results could be brought together at the end of the lesson to produce a comprehensive result.
- An extension activity can be to conduct the experiment on other prepackaged fruits and vegetables besides lettuce.
- Students can create posters on food safety.
- Students can research famous microbiologists such as Robert Koch or Louis Pasteur.
- Students can investigate case studies of foodborne illnesses.
- The same procedure can be done to test the effectiveness of other types of cleaning products or of handwashing.

Assessing the Lesson (steps not requiring teacher feedback are not listed below; see Student Instructions for complete step-by-step instructions)

Step 3 **Divide into groups of 3–5 students. Look at the wrapper from the bagged salad your teacher provides. Does the label say anything about whether the salad has been washed? Write the description below.**

Descriptions may vary, but examples could include "washed," "triple-washed," "ready-to-eat," or "needs to be washed."

Step 4 **If you wanted to wash this bagged salad before eating it, how would you wash it? Would you use cold or warm water? Would you use water only, dish soap, vinegar, or produce wash on the leaves? Would you rub each piece with your fingers, just toss it around with your hands under the running water, or just run it under water without any physical agitation with your hands? Would you soak it in water? How long would you wash it? Discuss as a group, and list the steps you would take to clean the salad. Be as specific as possible.**

Student answers will vary, but look for thoughtful, sequential, logical, and complete responses that address the questions listed.

Step 5b **Select 1 factor you listed in Step 4 (for instance, length of time) that you would like to test. This independent variable should have at least 2 experimental conditions. For example, if you want to observe the effect of different washing times, you will need to select Time 1 (for instance, 10 seconds) and Time 2 (for instance, 20 seconds). List these variables on pages 2 and 3 of the Record Sheet. Once you have identified the variables you want to test, the other variables (such as how the leaves are washed, water temperature, etc.) should remain constant.**

Student answers will vary, but look for logical and creative responses. Answers should include 1 variable that has at least 2 experimental conditions. Some possible answers include the following:

- How is the salad washed? (soaked, washed with hands, etc.)
- How long is the salad washed? (10 seconds, 20 seconds, etc.)
- What is the salad washed with? (water, produce wash, lemon juice, vinegar, etc.)
- What temperature of water did you use to wash the salad? (hot, cold)

Step 6 **Describe your experiment below.**

Student answers will vary, but look for logical and creative responses. The following example answers are based on an experiment using length of washing time as the variable.

a. **Hypothesis:** If a longer washing time reduces the number of microbes on salad leaves, then the longer salad leaves are washed, the fewer microbes there will be.

b. **Control:** Unwashed salad leaf

c. **Independent Variable:** Time
   - **Variable 1:** 10 seconds
   - **Variable 2:** 20 seconds

d. **Dependent Variable:** Number of bacterial colonies

e. **Predictions:** More bacterial colonies will grow after washing salad leaves for 10 seconds compared with washing salad leaves for 20 seconds.

f. **How will you conduct the salad-washing portions of your experiment? (The sampling and culturing protocol is already provided for you in the Culturing Instructions handout.) List the steps you will follow below. Be as specific as possible.**

   Student answers will vary, but look for complete, logical, and thorough responses.

g. **What are potential sources of contamination? List the aseptic techniques you will follow to minimize contamination.**

   Since bacteria occur everywhere, there are many potential sources of contamination. Potential sources of contamination include laboratory equipment (glassware, culture media, etc.), the environment (water, countertops, etc.), and procedures (not washing hands, reusing the same equipment for different samples, etc.). Students should list several

aseptic techniques, such as cleaning their hands and workstations before and after conducting the experiment, wearing gloves, and using sterile equipment.

Step 7 **How will you be able to tell if one washing technique worked better than another? Describe the process you will use to answer that question. Be as specific as possible.**

Student answers will vary, but look for complete, logical, and thorough responses.

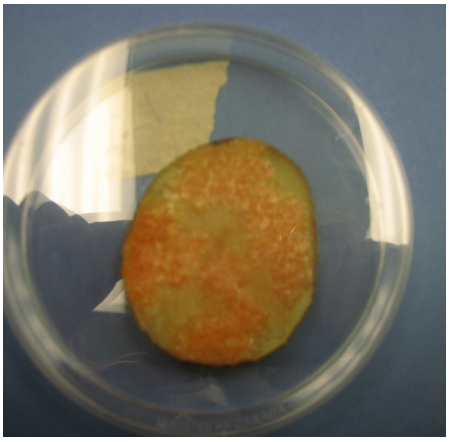
Step 8 **Conduct your salad-washing experiment, and follow the steps listed on the Culturing Instructions handout. When you have collected your samples, place them in the area indicated by your teacher. Then clean your workstation.**

Observe students' ability to follow instructions.

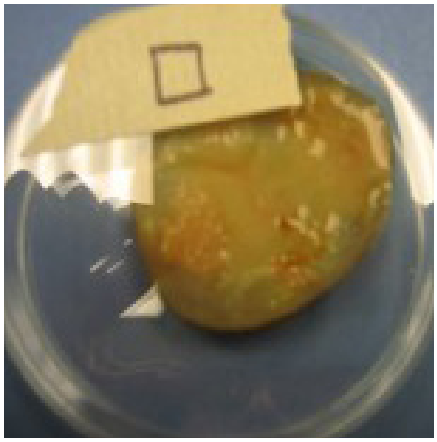
Step 10 **Look at any colonies growing on your cultures. Record your results on the Record Sheet, describing any growth you observe. Be sure to describe the number of colonies by counting each round spot, the size of the colonies, and the color and morphology of what you see. If there appear to be different-looking growths in the same sample, describe those growths and their differences. In your descriptions be sure to differentiate between your samples (Variable 1 versus Variable 2 versus your control).**

Look for complete and detailed responses on the Record Sheet. The following images show examples of potential results using potato slices:

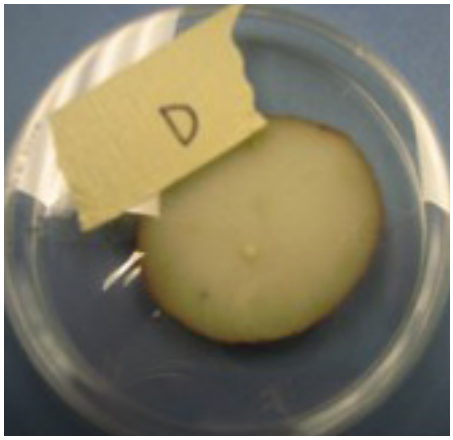
Control (unwashed salad leaf)



Salad leaf soaked in water for 20 seconds



Salad leaf soaked in lemon juice for 20 seconds



Step 11 **Based on your experiment, can you make any conclusions about the variable you tested? Summarize your results below. Did your observations match your predictions?**

Student answers will vary, but look for complete, logical, and thorough responses. Students should relate their observations back to the predictions and not overstate their results.

Step 12 **What changes would you make to improve your experiment? Describe the changes, and explain why you would make them.**

Student answers will vary, but look for complete, logical, and thorough responses.

Step 13 **Present your findings to the class. Be sure to include what variables you used and what your results were. Take notes on other people's washing tests and results.**

Presentations should include students' hypotheses, variables, predictions, observations, and conclusions.

Step 14 **Based on the results of the class's experiments, what are your recommendations for using prepackaged salads at home?**

Student answers will vary, but look for complete, logical, and thoughtful responses that do not overstate the data. Students should also be careful not to overstate the possible conclusions; unless each group uses salad from a different store, the sample size is too small to draw any firm conclusions.

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Give us your feedback! Send comments about this lesson to ehpscienceed@niehs.nih.gov.

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STUDENT INSTRUCTIONS:

Triple Washed, Twice Shy?

Step 1 Read the article "Triple Washed, Twice Shy?"

Step 2 Read the information below.

In the United States, approximately 76 million cases of foodborne disease are reported each year. These cases involve foods ranging from fresh produce to iced tea. Most cases of foodborne illness are not severe; patients may experience symptoms for 24–48 hours, and many cases likely go unreported. Foodborne disease is caused when people eat food that has been contaminated with pathogens, microorganisms that can cause disease.

Food may be contaminated with pathogens at several points in the production and packaging processes. For example, fruits and vegetables may become contaminated if they are grown with soil, water, or fertilizer that contains pathogens. Contamination can occur during packaging or preparation for sale if the food is handled by infected individuals (for instance, people who are sick can transfer the pathogen to the food if they do not wash their hands after using the restroom) or if the food is washed with contaminated water. Foods can also be contaminated in the kitchen. For instance, if you use the same cutting board or knife to prepare a salad and raw chicken, any pathogens in the chicken can contaminate the salad. Depending on how an item is washed, pathogens that are present on the produce may not be completely removed.

Most prepackaged salads have been commercially washed and packaged in sealed bags. These salads typically include the labels "washed," "triple-washed," or "ready-to-eat." The current assumption is that salads with these labels need not be washed before eating unless the package specifically mentions the need to rewash.

Step 3 Divide into groups of 3–5 students each. Look at the wrapper from the bagged salad your teacher provides. Does the label say anything about whether the salad has been washed? Write the description below.

Step 4 If you wanted to wash this bagged salad before eating it, how would you wash it? Would you use cold or warm water? Would you use water only, dish soap, vinegar, or produce wash on the leaves? Would you rub each piece with your fingers, just toss it around with your hands under the running water, or just run it under water without any physical agitation with your hands? Would you soak it in water? How long would you wash it? Discuss as a group, and list the steps you would take to clean the salad. Be as specific as possible.

National Institute of Environmental Health Sciences

Step 5 Read the information below, and then complete Steps 5a and 5b.

You will be conducting an experiment to see if washing prepared salads affects the number of microorganisms present on the salad leaves. This experiment uses the process of culturing. Culturing is a laboratory technique used to identify and study different microorganisms. In a foodborne illness outbreak, scientists may use cultures to identify the cause of disease.

To find out whether microorganisms are present in the salad, you will culture, or grow, samples of microbes. To prepare a culture, a sample is taken and isolated on a culture medium within a Petri dish. A culture medium is a substrate that contains all the nutrients necessary for microbial growth. Agar, made from seaweed, is the most commonly used culture medium. Other types of culture media include potatoes and bread.

When culturing for bacteria, it is important to make sure that any bacteria growing on your culturing medium are from your samples and not from other sources. Microorganisms are everywhere, and if you are not careful your sample could easily be contaminated from bacteria present on your hands, on your desk, or even in the air. Because you are interested only in the microorganisms present on the salad, you will need to follow aseptic techniques to conduct the experiment. Aseptic techniques involve taking precautions such as wearing gloves and using sterile (free from microorganisms) equipment to minimize contamination of your sample. Aseptic techniques include using disinfectants such as rubbing alcohol or bleach to clean laboratory surfaces, using presterilized products that are in sealed packages, scrubbing your hands vigorously with special soap and hot running water for at least 15 seconds, and heating your materials to a specific temperature for a sufficient length of time to sterilize them (for instance, sticking tweezers in a flame for a moment or boiling them for 30 minutes).

Aseptic techniques are also important to protect researchers from being exposed to potential pathogens that are present in many types of microbiological experiments. Treat your cultures as potential pathogens because harmful microorganisms, such as *Salmonella*, may be present on the produce or in the cultures. Follow the safety guidelines listed below:

- Do not open Petri dishes once they are closed.
- Wash your hands before and after doing any laboratory work, even if you wore gloves during the experiment.
- Do not bring food or drinks into the laboratory.
- Wear safety gloves and goggles.

a. Read the handout titled Culturing Instructions to learn about the culturing process.

b. Select 1 factor you listed in Step 4 (for instance, length of time) that you would like to test. This independent variable should have at least 2 experimental conditions. For example, if you want to observe the effect of different washing times, you will need to select Time 1 (for instance, 10 seconds) and Time 2 (for instance, 20 seconds). List these variables on pages 2 and 3 of the Record Sheet. Once you have identified the variables you want to test, the other variables (such as how the leaves are washed, water temperature, etc.) should remain constant.

Step 6 Describe your experiment below.

a. Hypothesis:

b. Control:

c. Independent Variable:

- Variable 1:

- Variable 2:

d. Dependent Variable:

e. Predictions:

f. How will you conduct the salad-washing portion of your experiment? (The sampling and culturing protocol is already provided for you in the Culturing Instructions handout.) List the steps you will follow below. Be as specific as possible.

g. What are potential sources of contamination? List the aseptic techniques you will follow to minimize contamination.

Step 7 How will you be able to tell if one washing technique worked better than another? Describe the process you will use to answer that question. Be as specific as possible.

Step 8 Conduct your salad-washing experiment, and follow the steps listed on the Culturing Instructions handout. When you have collected your samples, place them in the area indicated by your teacher. Then clean your workstation.

Step 9 After a few days of culturing, you will begin to see microbial growth. Read the following information about how researchers identify different bacteria that grow on cultures.

Bacteria tend to grow in colonies, or clusters, which look different depending on the type of bacteria. Researchers use many different methods to identify the type of bacteria they see growing in a culture. Researchers describe colonies based on such factors as their shape, color, and elevation. Colony shapes (or morphology) can be round, irregular, or filamentous (threadlike). Colony elevation can be raised or flat. Colonies can also appear rough or smooth, shiny or dull. Finally, colonies can be clear or opaque, and they come in many colors. Researchers also use microscopes to closely look at a colony's characteristics and structure.

In addition to looking at the colony's characteristics, individual bacteria can be identified based on cellular physical properties such as the shape of the cell (for instance, rod, spiral, or sphere shaped) and the structure of the cell walls

(for instance, thick as opposed to thin). Gram staining is a lab procedure used to identify gram-positive bacteria (which have thick cell walls) and gram-negative bacteria (which have thin cell walls). During gram staining, a violet dye is applied to bacterial colonies. The violet dye stains cell walls a dark purple color. The colonies are then washed with alcohol. Colonies with thick cell walls stay purple, while those with thinner cell walls lose their stain. A second counter-stain of red dye is then added. Any bacteria that lost their purple stain will now be dyed a pink or reddish color. After conducting a gram stain, gram-positive bacteria appear purple while gram-negative bacteria appear pink.

Bacteria can also be identified based on the culture medium on which they are grown. Selective media contain ingredients that support the growth of one type of microorganism over another. For example, MacConkey agar inhibits the growth of gram-positive bacteria. Differential media distinguish among different types of bacteria. Blood agar is used to identify bacteria that are hemolytic, which means they destroy red blood cells. Bacteria that destroy red blood cells have a clear area around their colonies.

Below are some descriptions of common foodborne pathogens:

- *Escherichia coli*: gram negative; rod shaped with flagella.
- *Clostridia*: gram positive, rod shaped. NOTE: The foodborne illness known as botulism is caused by a toxin produced by *C. botulinum*, not by the bacterium itself.
- *Salmonella*: gram negative; rod shaped with flagella; colonies are white, round, and smooth, approximately 2–4 mm in diameter.
- *Listeria*: gram positive; rod shaped; can grow in short chains (seen under microscope); flagella at room temperature; hemolytic colonies on blood agar.

Step 10 Look at any colonies growing on your cultures. If you used potato slices as your culture medium, then look at the potato-only control your teacher set up. Record your results on the Record Sheet, describing any growth you observe. Be sure to describe the number of colonies by counting each round spot, the size of the colonies, and the color and morphology of what you see. If there appear to be different-looking growths in the same sample, describe those growths and their differences. In your descriptions be sure to differentiate between your samples (Variable 1 versus Variable 2 versus your control).

You will not be identifying the specific type of bacteria (or yeast) growing in your culture, but knowing that there are differences between the appearance of the colonies is useful and will let you know if you have more than one type of microbe growing in your sample. When you have recorded all your data, give the cultures to your teacher for disposal.

Step 11 Based on your experiment, are you able to make any conclusions about the variable you tested? Summarize your results below. Did your observations match your predictions?

Step 12 What changes would you make to improve your experiment? Describe the changes, and explain why you would make them.

Step 13 Present your findings to the class. Be sure to include what variables you used and what your results were. Take notes on other people's washing tests and results.

Step 14 Based on the results of the class's experiments, what are your recommendations for using prepackaged salads at home?

Culturing Instructions

1. Begin by cleaning your work area with a disinfectant provided by your teacher. Wash your hands using soap and warm water for at least 30 seconds.

2. Your teacher will provide the following materials:
   - safety goggles and gloves for each group member
   - 1 bag of 6–8 sterile cotton swabs
   - 1 bag of 6–8 salad leaves
   - grease pencil or marker
   - masking tape
   - stopwatch
   - sterile tweezers
   - 1 clean plastic cup or beaker with water from your teacher
   - 3–6 sterile Petri dishes with the culture medium prepared by your teacher
   - paper towels
   - additional materials for washing salad leaves, if appropriate for your experiment

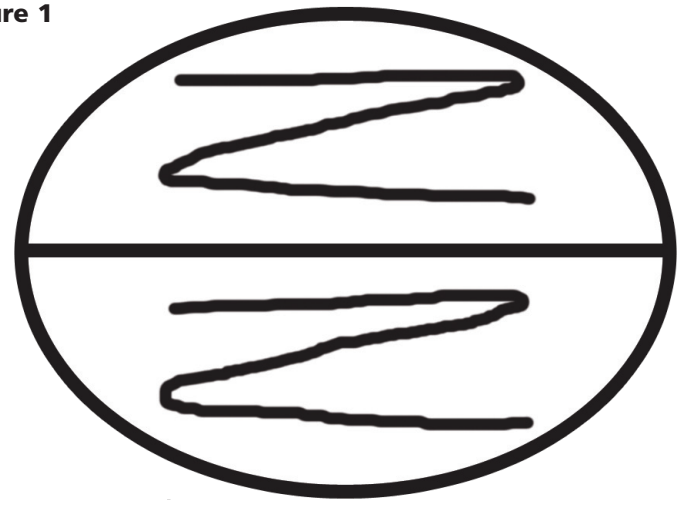
3. Label each Petri dish with the experimental condition and your group number/name. NOTE: If you are using agar, you will need to divide each Petri dish into 2 halves by either visualizing a line across the dish or marking with a grease pencil; 1 sample will go on each half of the dish. If you are using potatoes, you may need to use a separate dish for each control sample, depending on the size of the potato slices.

4. Put on safety gloves and/or goggles.

Sample Collection

5. Control: Open the bag of salad leaves. Use tweezers to select 1 leaf. This will be your control. Dip one end of a cotton swab into the cup of water. Rub the wet end over both sides of the leaf for Control, Sample C-1. Roll the cotton swab on the surface of the culture medium in the Petri dish in a zigzag pattern in half of the Petri dish, as seen in Figure 1. Select a second leaf and repeat for Control, Sample C-2, in the other half of the Petri dish or in a new dish. After finishing, close the Petri dish(es). Put the 2 leaves aside on the paper towel.

Figure 1



Culturing Instructions

6. Test 1: Use tweezers to select a new leaf. Wash the leaf using the methods you described in your protocol for Test 1, Sample 1-1. Dip one end of a new cotton swab into the cup of water. Rub the wet end over both sides of the salad leaf. Roll the cotton swab on the surface of the culture medium in a zigzag pattern. Repeat the same procedure for Test 1, Sample 1-2, in the other half of the Petri dish or in a new dish. After finishing, close the Petri dish(es). Put the leaves aside on the paper towel.

7. Test 2: Use tweezers to select a new leaf. Wash it using the methods described in your protocol for Test 2, Sample 2-1. Dip one end of a new cotton swab into the cup of water. Rub the wet end over both sides of the leaf. Roll the cotton swab on the surface of the culture medium in a zigzag pattern. Repeat the same procedure for Test 2, Sample 2-2, in the other half of the Petri dish or in a new dish. After finishing, close the Petri dish(es). Put the leaves aside on the paper towel.

9. Tear off a long piece of masking tape and lay it sticky side up on a table.

10. Stack your group's Petri dishes on the tape, and fold the edges of the tape over the dishes, forming a band around the dishes.

11. Write your group's name/number on the tape.

12. Let your plates sit at room temperature. Do not open the dishes.

13. Throw out all trash. Clean your workstation. Remove gloves and goggles, and wash your hands.

Preparing a Wet-Mount Slide

1. Collect the following materials:
    - 1 microscope
    - 1 clean microscope slide
    - 1 coverslip
    - 1 pipette
    - 1 small cup of water
    - 2 sterile probes or toothpicks
    - paper towels
    - pencil
    - safety gloves and goggles.
2. Put on the safety gloves and goggles.
3. Decide which colony you want to look at, and open the Petri dish. Gently rub the end of the sterile probe or toothpick in the selected colony. Close the Petri dish.
4. Gently rub the specimen on the middle of the microscope slide.
5. Use the pipette to obtain a drop of water. Place the drop of water on the specimen.
6. Carefully lower one edge of the coverslip so it touches the drop of water at approximately a 45° angle. Use a probe to slowly lower the rest of the coverslip on the specimen.
7. Remove any air bubbles by tapping the surface of the coverslip with a pencil eraser.
8. Use the paper towel to remove any excess water at the edge of the coverslip. If the specimen begins to dry out, add additional drops of water as needed.
9. Examine the specimen under a microscope. Be sure to record the magnification level you are using. Draw what you see below:

Record Sheet
Control

Observations			
Sample	**Day**	**Number of Colonies**	**Description of Colonies**
C-1	**3**		
	4		
	5		
	6		
C-2	**3**		
	4		
	5		
	6		
Potato-only control (if applicable)	**3**		
	4		
	5		
	6		

Record Sheet

Test 1

Observations			
Sample	**Day**	**Number of Colonies**	**Description of Colonies**
1-1	**3**		
	4		
	5		
	6		
1-2	**3**		
	4		
	5		
	6		

Record Sheet

Test 2

Observations			
Sample	**Day**	**Number of Colonies**	**Description of Colonies**
2-1	3		
	4		
	5		
	6		
2-2	3		
	4		
	5		
	6		